

Patent Claims

1. A method for the detection of cytosine methylation in
5 DNA characterized in that the DNA to be investigated
is brought into contact with a triplex-forming mole-
cule which distinguishes between methylated and un-
methylated DNA.
- 10 2. The method according to claim 1, further character-
ized in that the triplex-forming molecule forms a
triplex with the DNA to be investigated, whereby tri-
plex formation with unmethylated DNA is preferred
15 over triplex formation with methylated DNA, and the
triplex formation is used for the detection of the
methylation status.
- 20 3. The method according to claim 1, further character-
ized in that oligonucleotides, peptide nucleic acid
(PNA) oligomers, other oligonucleotide analogs or
chimeras, or molecules derived from these classes of
substance are used as the triplex-forming molecules.
- 25 4. The method according to one of claims 1 to 3, further
characterized in that the triplex-forming molecule
consists both a duplex-forming sequence as well as a
triplex-forming sequence.
- 30 5. The method according to one of claims 1 to 4, further
characterized in that the triplex-forming molecule
comprises at least one modified nucleobase, which
specifically or selectively binds to a cytosine in
the triplex.

6. The method according to claim 5, further characterized in that N⁴-substituted cytosine derivatives are used as nucleobases.
- 5 7. The method according to one of claims 5 to 6, further characterized in that N⁴-(3-acetamidopropyl)cytosine or N⁴-(6-amino-2-pyridinyl)cytosine is used as the nucleobase.
- 10 8. The method according to one of claims 5 to 7, further characterized in that N⁴-substituted cytosines, which comprise additional modifications at position 3, are used as nucleobases.
- 15 9. The method according to claim 8, further characterized in that position 3 is modified with a methyl, ethyl or isopropyl group.
- 20 10. The method according to one of claims 1 to 9, further characterized in that the triplex-forming molecule bears a detectable label.
- 25 11. The method according to one of claims 1 to 10, further characterized in that the methylation status is detected via an *in-situ* hybridization.
- 30 12. The method according to one of claims 1 to 10, further characterized in that for the detection of the methylation status, the DNA is amplified, wherein due to the triplex formation, the amplification of methylated DNA is preferred over the amplification of unmethylated DNA.
- 35 13. The method according to one of claims 1 to 10, further characterized in that for the detection of the methylation status, the DNA is amplified, wherein due

to the triplex formation, the amplification of unmethylated DNA is preferred over the amplification of methylated DNA.

- 5 14. The method according to one of claims 12 to 13, further characterized in that triplex-forming molecules are utilized, which also serve as primers in the amplification.
- 10 15. The method according to one of claims 1 to 12, further characterized in that structures which hinder amplification are formed by the triplex formation.
- 15 16. The method according to one of claims 12 to 15, further characterized in that deoxy-5-methylcytosine triphosphate, and not deoxycytosine triphosphate (dCTP), is utilized in the amplification.
- 20 17. The method according to one of claims 12 to 16, further characterized in that a real-time PCR is utilized for the amplification.
- 25 18. A method for the separation of methylated and unmethylated DNA characterized in that
- 30 (a) the DNA is brought into contact with a triplex-forming molecule,
- (b) the triplex-forming molecule forms a triplex with the DNA, wherein triplex formation with unmethylated DNA is preferred over triplex formation with
- (c) the triplex formation is utilized for the separation.
- 35 19. A method for the specific introduction of DNA damage into unmethylated DNA, characterized in that

(a) the DNA is brought into contact with a triplex-forming molecule which bears a reactive chemical group,

(b) the triplex-forming molecule forms a triplex with the DNA, wherein triplex formation with unmethylated DNA is preferred over triplex formation with methylated DNA,

(c) the reactive chemical group is reacted with the DNA present in triplex form.

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20. A method for the specific inhibition of replication of unmethylated DNA, characterized in that

(a) DNA is brought into contact with a triplex-forming molecule,

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(b) the triplex-forming molecule forms a triplex with the DNA, wherein triplex formation with unmethylated DNA is preferred over triplex formation with methylated DNA,

(c) the replication of the DNA present in triplex form is inhibited.

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21. A method for the specific inhibition of transcription of unmethylated DNA, characterized in that

(a) DNA is brought into contact with a triplex-forming molecule,

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(b) the triplex-forming molecule forms a triplex with the DNA, wherein triplex formation with unmethylated DNA is preferred over triplex formation with methylated DNA,

(c) the transcription of the DNA present in triplex form is inhibited.

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22. Use of oligonucleotides, peptide nucleic acid (PNA) oligomers, other oligonucleotide analogs or chimeras, or molecules derived from these substance classes, which contain N⁴-(3-acetamidopropyl)cytosine, N⁴-(6-

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amino-2-pyridinyl)cytosine or other N⁴-substituted cytosine derivatives, for the therapy of disorders which are associated with cytosine demethylation.